Supplementary figures

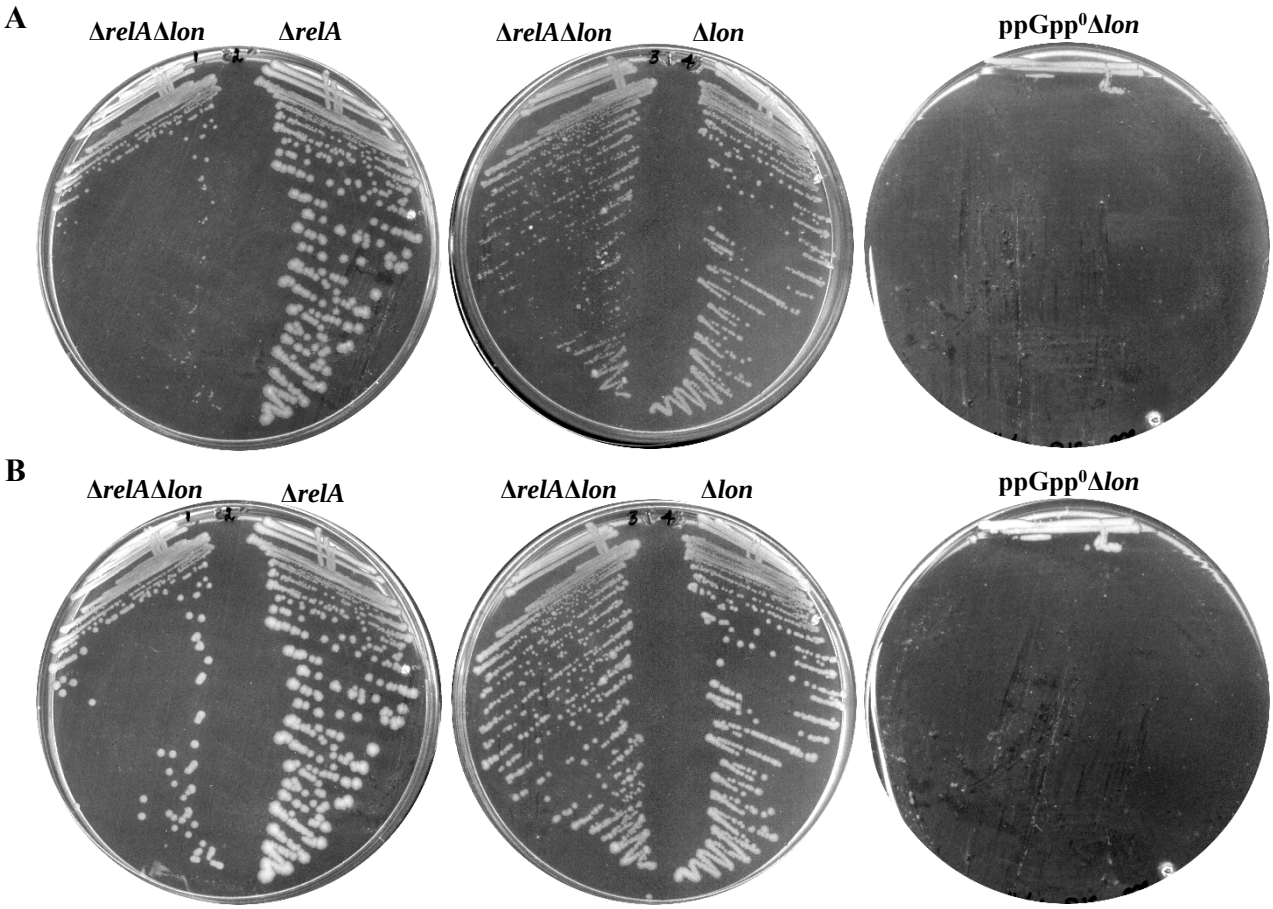

Fig. S1. Growth defect of strains in LB medium. Strains whose relevant genotypes are indicated were streaked and the plates photographed after 24 hours (A) or 40 hours (B) of incubation at 37°C. The ppGpp^0 Δ*lon*/ pRCspoT strain was maintained in the presence of Amp and IPTG.

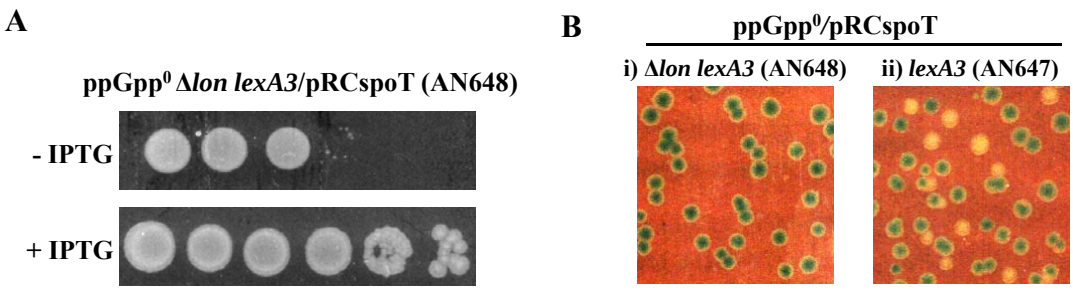

Fig. S2. The synthetic growth defect of ppGpp^0 Δ*lon* strain is not suppressed by *lexA3*.

Strains whose relevant genotypes are indicated were studied for plating defect (A) or the instability of plasmid pRCspoT (B) Plating defect was monitored in LB medium in the absence or presence of 1mM IPTG. Plasmid loss was monitored in LB medium containing 1mM IPTG and X-Gal.

Supplementary tables

Table S1. Co-transduction frequency of Δ*lon*::Kan and *tsx*::Tn*10*[a] markers by phage P1 transduction.

Strain	Relevant genotype	Number of Tet^R transductants screened	Number of Tet^R transductants that are Kan^R	co-transduction (%)
MG1655	Wild-type	263	38	14[b]
CF12510	Δ*relA*	305	60	19
HR202	Δ*relA* Δ*spoT*	270	0	<0.4
AN120[c]	Δ*relA* Δ*spoT* / pRCspoT	270	33	12

a- *tsx*::Tn*10* is linked 66% to lon^+ as deduced by introducing *tsx*::Tn*10* by phage P1 transduction into MG1655 Δ*lon*::Kan (AN207); 51 Tet^R Kan^S transductants were scored after screening 77 Tet^R transductants. P1 lysate prepared on an MG1655 *tsx*::Tn*10* Δ*lon*::Kan strain was used for performing the co-transduction experiments.

b- The reason for decrease in co-transduction frequency from the expected 66% is not apparent.

c- Transductants were selected and screened in the presence of Amp and IPTG.

Table S2. Quantification of pRCspoT plasmid instability.

Strain	Relevant genotype[a]	Frequency of plasmid loss (white colonies / total colonies)
AN131	Wild-type	**0.35 (54/156)**
AN207	Δ*lon*::kan	**0.46 (344/752)**
AN256	Δ*relA*::FRT	**0.26 (74/280)**
AN120	Δ*relA* Δ*spoT*	**0.21 (46/216)**
AN844	Δ*relA*::FRT Δ*lon*::Kan	**0.34 (167/492)**
AN519	Δ*relA* Δ*spoT* Δ*lon*::Kan	**<0.001 (0/952)**
AN567	Δ*relA* Δ*spoT* Δ*lon*::Kan Δ*sulA*::FRT	**0.23 (218/966)**
AN568	Δ*relA* Δ*spoT* Δ*lon*::Kan Δ*rcsB*::FRT	**<0.001 (0/873)**
AN512	Δ*relA* Δ*spoT* Δ*lon*::Kan / pBAD24	**<0.006 (0/175)**
AN513	Δ*relA* Δ*spoT* Δ*lon*::Kan / plon$^+$ (− arabinose)	**<0.003 (0/357)**
AN513	Δ*relA* Δ*spoT* Δ*lon*::Kan / plon$^+$ (+ arabinose)	**0.15 (139/916)**
AN514[b]	Δ*relA* Δ*spoT* Δ*lon*::Kan / plon S679A (+ arabinose)	**<0.004 (0/238)**
AN515[b]	Δ*relA* Δ*spoT* Δ*lon*::Kan / plon K362Q (+ arabinose)	**<0.005 (0/201)**
AN516[b]	Δ*relA* Δ*spoT* Δ*lon*::Kan / plon S679A K362Q (+ arabinose)	**<0.009 (0/106)**

AN825	Δ*dksA*::FRT Δ*lon*::Kan	**0.43 (176/410)**
AN688	Δ*relA* Δ*spoT* Δ*lon*::Kan / pdksA′	**<0.005 (0/221)**
AN689	Δ*relA* Δ*spoT* Δ*lon*::Kan / pdksA	**0.24 (72/304)**
AN690	Δ*relA* Δ*spoT* Δ*lon*::Kan / pdksA^{NN}	**0.2 (68/332)**
AN677	Δ*relA* Δ*spoT* Δ*lon*::Kan /pCL1920	**<0.001 (0/855)**
AN678	Δ*relA* Δ*spoT* Δ*lon*::Kan / pCLftsQAZ	**0.43 (148/348)**
AN679	Δ*relA* Δ*spoT* Δ*lon*::Kan / pCLsdiA	**0.41 (112/272)**
AN680	Δ*relA* Δ*spoT* Δ*lon*::Kan / pSCftsQAZ	**0.39 (192/488)**
AN792	Δ*relA* Δ*spoT* Δ*lon*::Kan / pCA24N	**<0.01 (0/95**
AN793	Δ*relA* Δ*spoT* Δ*lon*::Kan / pCA24NftsZ	**0.15 (8/52)**
AN840	Δ*relA* Δ*spoT* Δ*lon*::FRT attλ::(P_{208}-*ftsZ-gfp*) (+ 0.1mM IPTG)	**0.19 (68/364)**
AN647	Δ*relA* Δ*spoT lexA3 mal*::Tn*9*	**0.22 (67/311)**
AN648	Δ*relA* Δ*spoT* Δ*lon*::Kan *lexA3 mal*::Tn*9*	**<0.002 (0/646)**
AN750	Δ*relA* Δ*spoT* Δ*lon*::FRT Δ*recA*::Kan	**<0.002 (0/453)**

a - All strains studied carry the Δ*lacZYAI*::FRT allele on the chromosome and either plasmid pRCspoT (Amp^R) or pRC_{Sp}-spoT (Sp^R) except AN825 which carries pRCdksA (Amp^R). Segregation of blue and white colonies was followed in LB agar plates with IPTG and X-gal. Whenever a second plasmid was present in the strain appropriate antibiotic was added to select for the plasmid and whenever required inducer was added as indicated to activate expression of the plasmid borne gene.

b –frequency of plasmid loss was similar in the presence or absence of arabinose.

Table S3. Strains and plasmids.

Strain or plasmid	Relevant genotype or features	Source or reference
MG1655	F^- λ^- *ilvG*$^-$ *rfb-50 rph-1*, sequenced wild-type K12	Lab collection
AG1	*recA1 endA1 gyrA96 thi-1 hsdR17* ($r_K^- m_K^+$) *supE44 relA1*	(1)
JW0093	AG1 / pCA24NftsZ	(1)
AN56	MG1655 Δ*relA*::FRT	This study
HR202	MG1655 Δ*relA* Δ*spoT*	This study
AN131	MG1655 Δ*lac*::FRT / pRCspoT	This study
AN256	AN131 Δ*relA*::FRT	This study
AN120	HR202 Δ*lac*::FRT / pRCspoT	This study
AN207	AN131 Δ*lon*::Kan	This study
AN844	AN256 Δ*lon*::Kan	This study
AN519	AN120 Δ*lon*::Kan	This study
AN518	HR202 Δ*lac*::FRT Δ*lon*::Kan / pRC_{Sp}-spoT	This study
AN512	AN518 / pBAD24	This study
AN513	AN518 / plon$^+$	This study
AN514	AN518 / plonS679A	This study

AN515	AN518 / plonK362Q	This study
AN516	AN518 / plonS679A K362Q	This study
AN565	AN120 Δ*sulA*:: FRT	This study
AN567	AN565 Δ*lon*::Kan	This study
AN568	AN519 Δ*rcsB*::FRT	This study
AN647	AN120 *lexA3* (Ind⁻) *malB*::Tn*9*	This study
AN648	AN647 Δ*lon*::Kan	This study
AN750	AN120 Δ*lon*::FRT Δ*recA*::Kan	This study
AN658	MG1655 Δ*lac*::FRT *sulA-lacZ*	This study
AN837	AN658 *srl300*::Tn*10*	This study
AN838	AN837 *recA56 srl300*::Tn*10*	This study
AN839	AN658 *lexA3* (Ind⁻) *malB*::Tn*9*	This study
AN660	HR202 Δ*lac*::FRT *sulA-lacZ*	This study
AN774	AN658 Δ*lon*::FRT	This study
AN743	AN660 Δ*lon*::FRT	This study
AN803	MG1655 Δ*lac*::FRT Δ*sulA*::Kan	This study
AN825	MG1655 Δ*lac*::FRT Δ*dksA*::FRT Δ*lon*::Kan / pRCdksA	This study
AN688	AN518 / pdksA′	This study
AN689	AN518 / pdksA	This study

AN690	AN518 / pdksA^{NN}	This study
AN352	AN519 *rpoB*35 btuB*::Tn*10*	This study
AN353	AN519 *rpoB* L571P *btuB*::Tn*10*	This study
AN826	AN519 *btuB*::Tn*10*	This study
AN677	AN519 / pCL1920	This study
AN678	AN519 / pCLftsQAZ	This study
AN679	AN519 / pCLsdiA	This study
AN680	AN519 / pSCftsQAZ	This study
AN792	AN519 / pCA24N	This study
AN793	AN519 / pCA24NftsZ	This study
AN840	AN518 attλ::(P_{208}-*ftsZ-gfp*, Amp)	This study
AN819	HR202 Δ*lac*::FRT / plon^{+}	This study
Plasmids		
pCL1920	pSC101-based; Sp^{R}	(2)
pBAD24	ColE1-derived; Amp^{R}	(3)
pCP20	pSC101 temperature-sensitive replicon, Amp^{R}, Cm^{R}	(4)
pRC7	Mini-F based; Amp^{R}	(5)
pRCspoT	pRC7 with *spoT*	This study
pRCdksA	pRC7 with *dksA*	This study

pRC_{Sp}-spoT	*bla* of pRCspoT replaced with *aadA* gene; Sp^R	This study
$plon^+$	pBAD24 with lon^+	(6)
plonS679A	pBADlonS679A	(6)
plonK362Q	pBADlonK362Q	(6)
plonS679A K362Q	pBADlonS679A K362Q	(6)
pMN8	pCL1920-*ftsQAZ* (pCLftsQAZ)	(7)
pMN15	pCL1920-*sdiA* (pCLsdiA)	(7)
pTB63	pSC101-based carrying *ftsQAZ* (pSCftsQAZ); Tet^R	(8)
pCA24N	Cloning vector used in ASKA Library; Cm^R	(1)
pCA24NftsZ	pCA24N carrying His_6-*ftsZ*	(1)
pJK537	pBR322-dksA (pdksA); Amp^R	(9)
pHM1684	pBR322-$dkaA^{NN}$ ($pdksA^{NN}$), aspartic acid residues at 71 and 74 positions replaced with asparagine; Amp^R.	Mike Cashel
pHR53	pBR322-dksA′ (pdksA′), dksA deleted after 36^{th} amino acid; Amp^R	This study

Table S4. Primers used in the study.

Primer	**Sequence**
JGOpRC7spoTFP	ATGAATTCGTTAATCACAAAGCGGGTCG
JGOpRC7spoTRP	ATAAGCTTAATTTCGGTTTCGGGTGACT
JGOpRC7dksAFP	ATGAATTCGATAGTGCGTGTTAAGGAG
JGOpRC7dksARP	ATAAGCTTAGCCAGCCATCTGTTTTTCG
JGOspecpRChomFP	ACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGT ATGCGCTCACGTAACTGGTC
JGOspecpRChomRP	AATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAG TTATTTGCCGACTACCTTGG
JGOdellacZYAIFP	CACCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGA ATTCCGGGGATCCGTCGACC
JGOdellacZYAIRP	CGTATCAGGCAATTTTTATAATTTAAACTGACGATTCAACG TGTAGGCTGGAGCTGCTTCG

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